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A MORPHOLOGICAL ANALYSIS OF A HYBRID SWARM OF NATIVE *ULMUS RUBRA* AND INTRODUCED *U. PUMILA* (ULMACEAE) IN SOUTHEASTERN NEBRASKA

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ABSTRACT

The parental species and hybrid swarm of native *Ulmus rubra* Muhl. and the introduced, naturalized and weedy *U. pumila* L. were investigated in a 65-km transect in Lancaster, Saunders, and Butler counties in Nebraska. Thirty-two trees of *U. rubra*, 32 of *U. pumila*, and 50 of the hybrid swarm were sampled for leaves and buds and subsampled for flowers and fruits. Leaves were measured for petiole length, blade length, width, primary and secondary teeth per cm, number of secondary teeth per primary tooth, and texture. Buds were scored for color and distribution of trichomes. Flowers were sampled for stamen counts and pollen size. Fruits were measured for length, width, and color and distribution of trichomes. Statistically significant differences ($P<.05$) were detected between the two parental species in all characters except fruit lengths. The hybrid swarm was also significantly different from both parental species, except in such characters as leaf length-to-width ratios, numbers of teeth, petiole length, and pollen size. A formal description of the hybrid swarm is given, and its name is proposed as *Ulmus* × *intermedia* Elowsky.

KEY WORDS: *Ulmus* hybrids, introgression, invasive trees, flowering phenology

Ulmus pumila L., the Siberian elm, is a north Asian species introduced to North America by J. G. Jack of the Arnold Arboretum in 1905 (Wyman 1951). Frank N. Meyer of the United States Department of Agriculture (USDA) then introduced it as a shelterbelt tree in 1908 (Engstrom & Mathew 1942; Wyman 1951). Because it is adapted for dry conditions and can survive severe winters, agricultural research stations across the Great Plains suggested planting it throughout the area (Dickens et al. 1928). Since the early 1950s, *U. pumila* has lost much of its popularity and is now rarely planted, but it has escaped cultivation and become an abundant and troublesome weed in many places, including Nebraska (Stubbendieck et al. 1995; Kaul et al. 2011).

Ulmus rubra Muhl., the red or slippery elm, is a native tree with a range that extends from Nebraska east to the Atlantic Ocean, and from Ontario to southern Texas. In our area, most red elms die of Dutch elm disease (DED; *Ophiostoma ulmi, O. novo-ulmi*) and other diseases after a decade or two, but not before sexual maturity. Hence, despite the DED pandemic, it persists.

Naturally occurring putative hybrids of *Ulmus rubra* and *U. pumila* were noted in Lancaster County, Nebraska, in 1995 (Kaul 1995). This presumably introgressant hybrid swarm was informally called *Ulmus* × *notha* Wilhelm and Ware *ined.* (Swink & Gerould 1994) in Illinois; we are here formally naming it *U.* × *intermedia.* It exhibits various combinations of parental traits. A gradient may exist in these intermediate traits due to backcrossing with one or both parental species and to crossing of hybrids with each other, hence our investigations into the populations.

Ulmus × *intermedia* is known in Iowa, Minnesota, Kansas, South Dakota, Texas, Nebraska, Illinois, and probably elsewhere (Kaul et al. 2011). Natural hybridization may have been occurring in our study area for more than half a century because the hybrid was first reported elsewhere in 1950 (Anonymous 1950). Nearly fifty years passed before an attempt was made to describe it and note the variation within the hybrid swarm (Swink & Gerould 1994; Kaul 1995). A full description of the morphological characters that define *U.* × *intermedia* has not been provided until now. The horticulture industry has made artificial crosses of these species in an attempt to create more desirable landscape trees having DED resistance (e.g., Smaller & Guries 1993); such hybrids are grown as named cultivars (Santamour & Bentz 1995).

Publications exist for the hybrids, (e.g., Kaul 1995; Swink & Gerould 1994; Zalapa et al. 2008a, 2009, 2010; Schlautman et al. 2012), but information in the horticultural and arboricultural literature is primarily about the artificial hybrids and mostly concerns DED and the production of resistant selections (e.g., Santamour 1993; Smalley & Guries 1993; Zalapa et al. 2008b; Santini et al. 2012). A direct correlation exists between the percentage of *Ulmus rubra* genes and susceptibility to DED, wherein 25 percent or greater *U. rubra* genes makes the trees highly susceptible to DED (Lester and Smalley 1972a). In a later paper (Lester & Smalley 1972b), a relationship was identified that links *U. rubra* genes to leaf size, lateness of flushing, tree height, stem dieback, and susceptibility to DED in the hybrids. Subsequent studies show similar results in which higher percentages of *U. rubra* genes increase susceptible to DED (Santamour 1974; Townsend & Schreiber 1976). The studies by Zalapa et al. (2008a, 2009) addressed hybridization between these species and tracked the patterns of introgression, which they found to be present from both parents but biased toward *U. pumila.* In further investigation of *U. pumila* genetic diversity, Zalapa et al. (2010) found little variation in the genetic population structure of naturalized mature stands and native Asian habitats. They did find *U. rubra* to be hybridizing into these natural stands and it is strongly visible in the microsatellite STRUCTURE results (Zalapa et al. 2009, 2010).

Complementary to the genetic marker analyses cited above, our goal here is to identify the gradient of morphological characters between parents and presumed hybrids in a local interbreeding population in southeast Nebraska, to use these characters to delineate the hybrid swarm, and to name and describe *U.* × *intermedia.*

MATERIALS AND METHODS

Leaves, buds, flowers, pollen, and fruits of *Ulmus rubra*, *U. pumila* and *U.* × *intermedia* were sampled in Lancaster, Butler, and Saunders counties, Nebraska, through three springs and summers (Fig. 1, Appendix 1). Representative specimens from the 13 sites are deposited in the University of Nebraska–Lincoln's Bessey Herbarium as NEB 318091–318108. Leaf and bud samples were collected in autumn, when buds are fully formed, for a total of 114 trees, with samples from 32 *U. rubra*, 32 *U. pumila,* and 50 *U.* × *intermedia.* Samples were taken entirely from sunny exposures to control for variation in sun- and shade-leaf morphology. Mature shoots with floral buds were chosen because their leaves are less variable than those of sucker shoots and leaders, which lack buds. Scanning electron micrographs (SEM) were produced with a Cambridge Stereoscan 90 to demonstrate differences in trichome density and size from fresh leaves that were collected in May.

Fresh leaves were placed directly into the microscope without critical-point drying and sputter coating, as per methods outlined in Lee (1984).

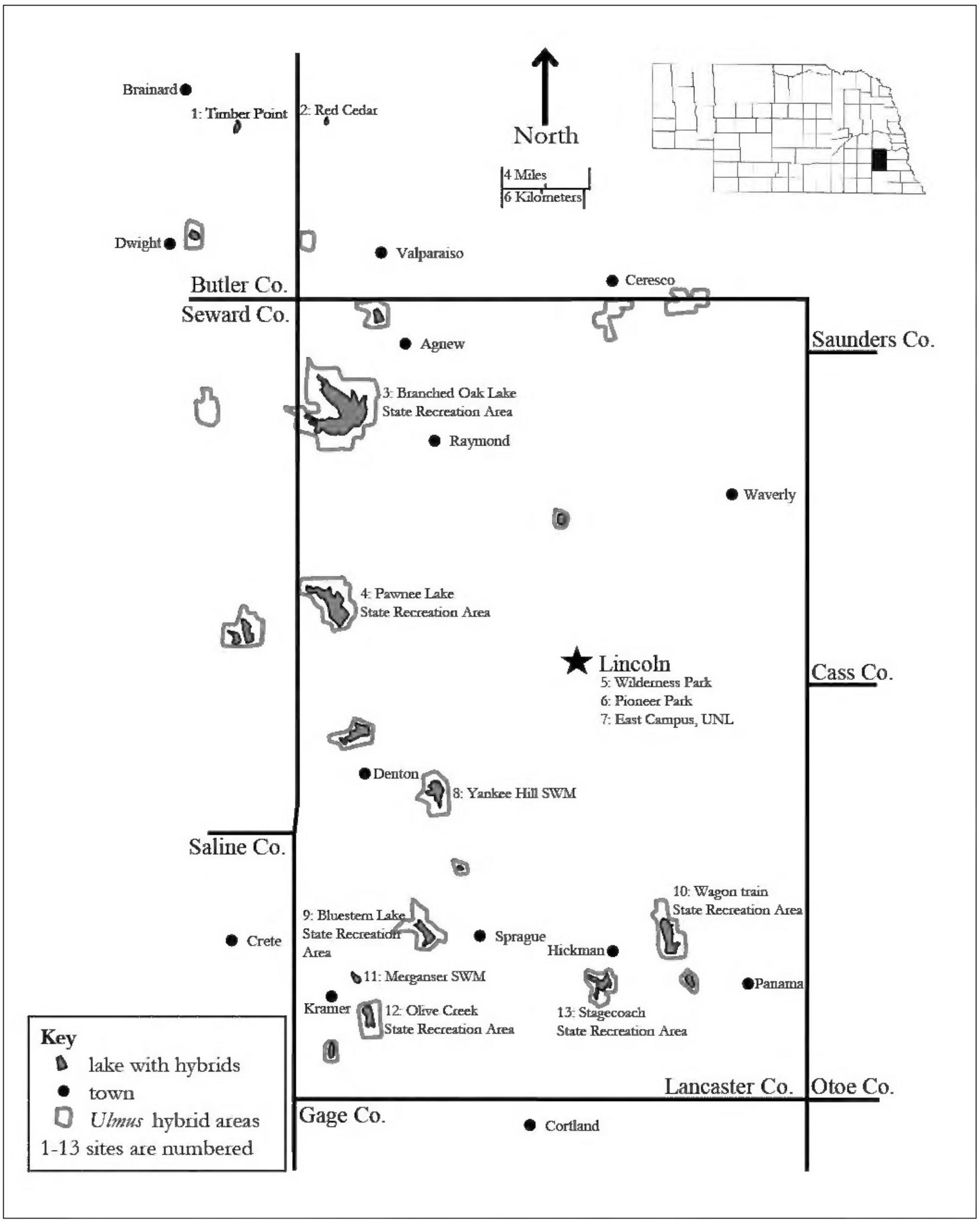
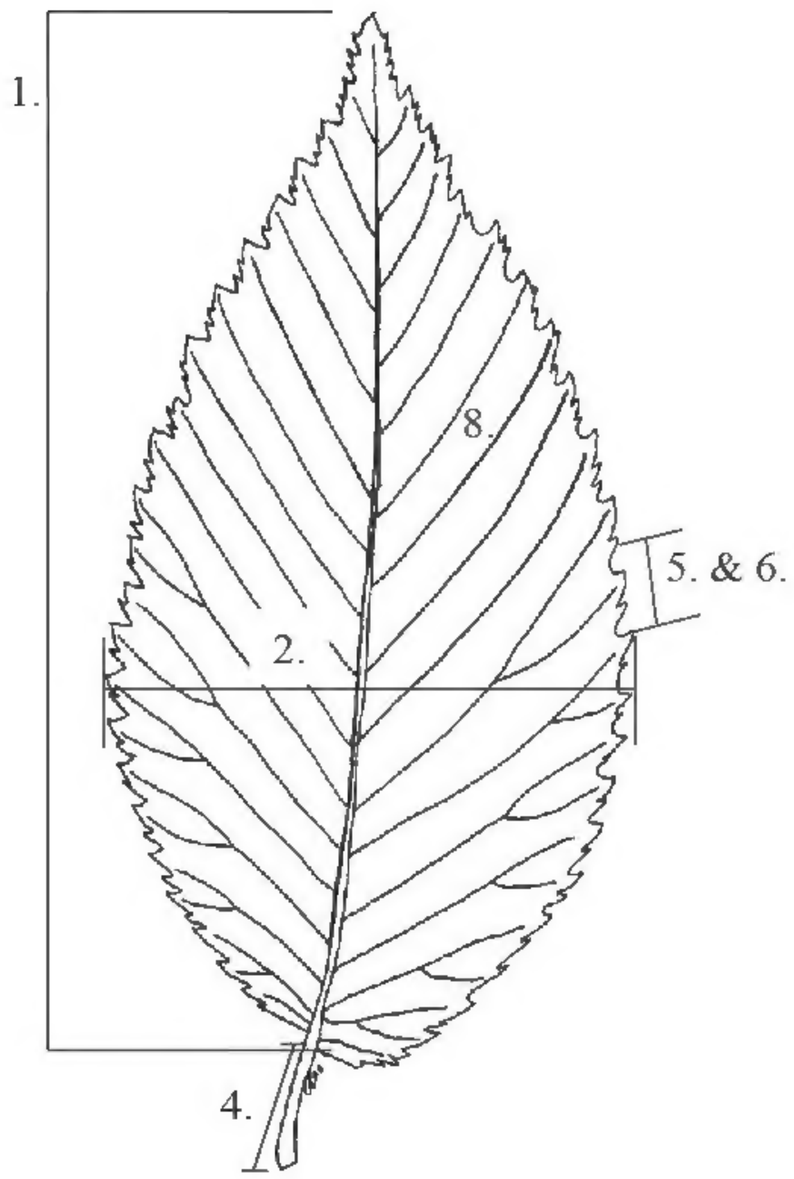


Figure 1. Nebraska area map of 13 sampling areas and sites for leaves, buds, stamens, pollen, or fruits of *Ulmus rubra*, *U.* × *intermedia*, and *U. pumila* (grey outlines refer to area around a body of water with *Ulmus* habitat).

One leaf was selected to represent each sampled tree. The second leaf from the apex of the shoot was selected for all quantitative and qualitative measurements (Fig. 2, Table 1). Morphometrics similar to those used in other *Ulmus* studies (Collins 1967; Nilsson 1980; Sherman 1987; Jeffers 1999) were chosen for this study. All leaves were measured for length, greatest width, and petiole length. The numbers per centimeter were counted for primary and secondary teeth and denticles, from the widest point of the leaf on the longer side of the leaf for 1 centimeter, moving apically. The ratio of length to width and number of secondary teeth per primary tooth per centimeter were calculated. The final foliar character scored was the texture of the adaxial surface. A score of (1) for scabrous (rough) or a score of (0) for glabrous (smooth) was assigned for every leaf, based on the force required to slide a finger across the surface. If the petiole of the sampled leaf flexed before the sample slid, a score of (1) was assigned, and those with a score of (0) slid without movement of any kind in the petiole. Scorings of the floral buds on the same shoot as the leaf samples were based upon two measures, distribution and color of trichomes (Table 1). Bud scales with nearly or only marginal trichomes were scored as (0), and those with over 1/3 of the scale covered, from the margin moving basipetally, were scored as (1). Bud scales with only white trichomes were scored as (0), and those with both rufous and white trichomes were scored as (1).



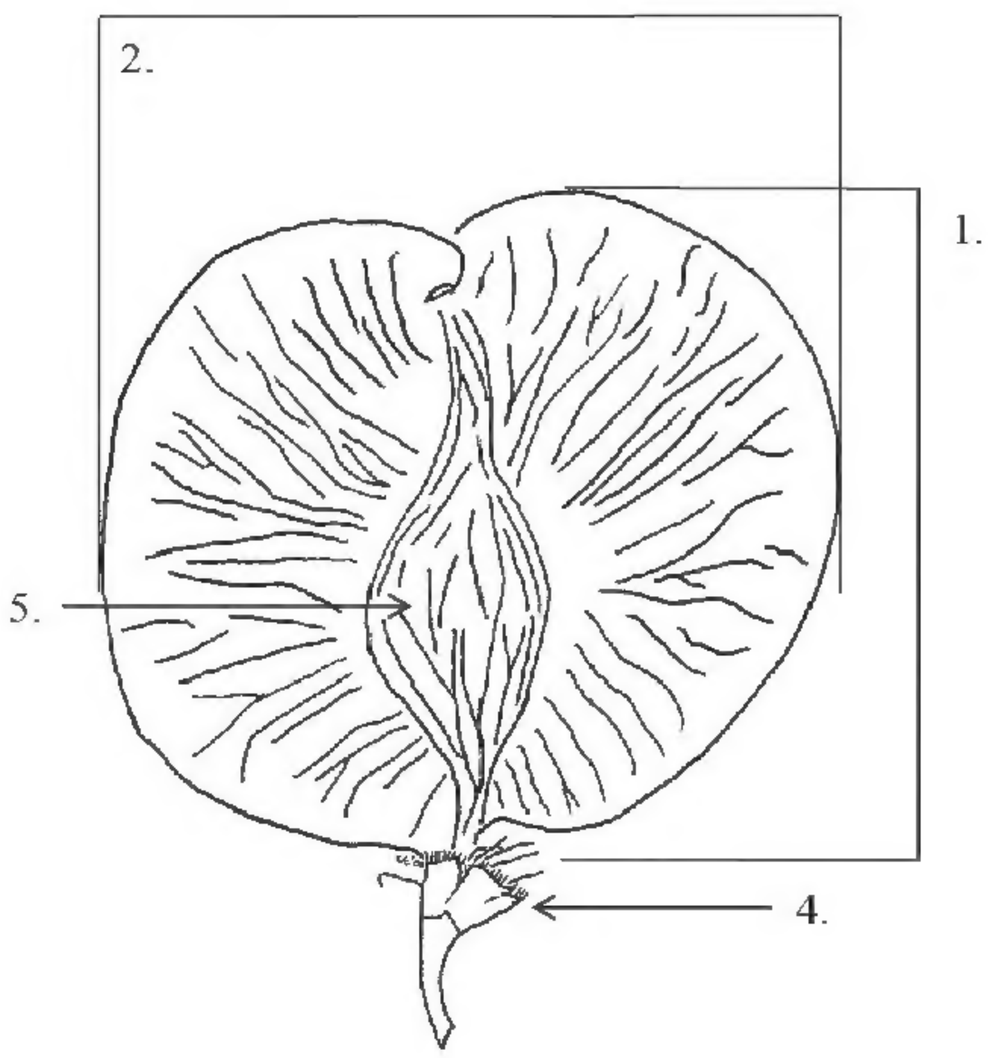


Figure 2 (left). Qualitative and quantitative measures made on leaves. a) Length of lamina from apex to petiole attachment; b) width of lamina at widest point; c) length / width ratio; d) petiole length from lamina attachment to stem; e) number of primary teeth from the widest point of the blade moving apically for 1 cm; f) number of secondary teeth from the widest point of the leaf moving apically for 1 cm; g) ratio of secondary teeth per primary tooth per cm counted from the widest point in the blade, moving apically for 1 cm; h) texture of adaxial surface, scored as scabrous or glabrous.

Figure 3 (right). Qualitative and quantitative measures made on fruits. a) Length of samara from lowest lobe to calyx; b) width of samara at widest point; c) length / width ratio; d) color of trichomes on persistent calyx; e) Presence or density of trichomes on fruit wall over seed.

Table 1. *Ulmus* foliar quantitative and qualitative characters measured for the three species. x= least squares mean and s= standard error from General Linear Model (GLM). P value from GLM and Chi-Square tests (*r*)= *U. rubra*, (*x*)= *U.* × *intermedia* and (*p*)= *U. pumila.*

	U. rubra	***U.* × *intermedia***	***U. pumila***	***P*≤value**
	x±s	**x±s**	**x±s**	
Foliar	**N=32**	**N=50**	**N=50**	
1. Length of lamina (cm)	12.22±0.31 range 8.5–16.6	8.58±0.24 range 4.7–11.7	5.16±0.31 range 4.0–7.2	*r*:*p* 0.0001 *x*:*r* 0.0001 *x*:*p* 0.0001
2. Width of lamina (cm)	6.27±0.14 range 4.0–8.2	4.16±0.11 range 2.6–5.8	2.30±0.14 range 1.7–3.2	*r*:*p* 0.0001 *x*:*r* 0.0001 *x*:*p* 0.0001
3. Length / width ratio	1.96±0.05 range 1.4–2.6	2.09±0.04 range 1.2–2.8	2.26±0.05 range 1.8–2.9	*r*:*p* 0.0001 *x*:*r* 0.0563 *x*:*p* 0.0094
4. Petiole length (cm)	0.78±0.03 range 0.5–1.1	0.81±0.03 range 0.3–1.2	0.56±0.03 range 0.3–0.9	*r*:*p* 0.0001 *x*:*r* 0.4396 *x*:*p* 0.0001
5. Primary teeth / cm	1.91±0.11 range 1.0–3.0	2.1±0.09 range 1.0–4.0	2.7±0.11 range 2.0–4.0	*r*:*p* 0.0001 *x*:*r* 0.1849 *x*:*p* 0.0001
6. Secondary teeth / cm	3.44±0.19 range 2.0–6.0	4.0±0.15 range 1.0–7.0	2.5±0.19 range 0.0–4.0	*r*:*p* 0.0011 *x*:*r* 0.0235 *x*:*p* 0.0001
7. Ratio of secondary to primary teeth / cm	2.13±0.18 range 0.8–5.0	2.12±0.14 range 0.5–6.0	0.98±0.18 range 0.0–1.4	*r*:*p* 0.0001 *x*:*r* 0.9771 *x*:*p* 0.0001
8. Texture of adaxial surface	Scabrous (1)	Either condition (1)=21 (0)=29	Glabrous (0)	*r*:*p* 0.0001 *x*:*r* 0.0001 *x*:*p* 0.0001
Buds	**N=32**	**N=50**	**N=50**	
9. Distribution of trichomes on bud scales	Nearly entire (1)	Either condition (1)=38 (0)=12	Marginal only (0)	*r*:*p* 0.0001 *x*:*r* 0.0001 *x*:*p* 0.0001
10. Color of trichomes on scales	Rufous and white (1)	Either condition (1)=45 (0)=5	White (0)	*r*:*p* 0.0001 *x*:*r* 0.0001 *x*:*p* 0.0001

Table 2: *Ulmus* floral and fruit quantitative and qualitative characters measured for the three species. x= least squares mean and s= standard error from General Linear Model (GLM). P value from GLM and Chi-Square tests (*r*)= *U. rubra*, (*x*)= *U.* × *intermedia* and (*p*)= *U. pumila.*

	U. rubra	***U.* × *intermedia***	***U. pumila***	***P*≤value**
Stamens	**x±s N=256 from 6 trees**	**x±s N=947 from 22 trees**	**x±s N=191 from 5 trees**	
#/floret from 2 buds/tree	7.08±0.04 Counts: 6 = 59 7 = 126 8 = 63 9 = 8	4.98±0.02 Counts: 4 = 188 5 = 599 6 = 157 7 = 3	4.10±0.04 Counts: 3 = 4 4 = 166 5= 21	*r*:*p* 0.0001 *x*:*r* 0.0001 *x*:*p* 0.0001
Pollen	**x±s N=75** 15 / 5 trees	**x±s N=150** 15 / 10 trees	**x±s N=75** 15 / 5 trees	
Width (µm)	32.59±0.29 Range 27.0–41.9	29.00±0.20 Range 23.2–35.0	29.67±0.29 Range 21.9–35.0	*r*:*p* 0.0001 *x*:*r* 0.0001 *x*:*p* 0.0575
Fruits	**x±s N=50 from 10 trees**	**x±s N=100 from 20 trees**	**x±s N=50 from 10 trees**	
1. Length (mm)	14.17±0.24 range 10.0–16.8	16.07±0.17 range 11.5–21.0	14.73±0.24 range 12.2–18.9	*r*:*p* 0.0971 *x*:*r* 0.0001 *x*:*p* 0.0001
2. Width (mm)	10.31±0.25 range 6.5–12.8	14.29±0.18 range 10.0–20.0	11.92±0.25 range 9.8–14.8	*r*:*p* 0.0001 *x*:*r* 0.0001 *x*:*p* 0.0001
3. Length / width ratio	1.40±0.02 range 1.1–2.1	1.13±0.01 range 0.9–1.5	1.24±0.02 range 1.0–1.6	*r*:*p* 0.0001 *x*:*r* 0.0001 *x*:*p* 0.0001
4. Color of trichomes on persistent calyx	Rufous and white (1)	Either condition (1)=70 (0)=30	White (0)	*r*:*p* 0.0001 *x*:*r* 0.0001 *x*:*p* 0.0001
5. Trichomes present over seed	Trichomes dense and extend to wings of samara (2)	Sparse trichomes on fruit wall (1) or Essentially glabrous (0) (2)=0 (1)=40 (0)=60	Essentially glabrous (0)	*r*:*p* 0.0001 *x*:*r* 0.0001 *x*:*p* 0.0001

In February of two years, branches were cut and flowers were forced in tap water in the laboratory. Flowers were placed in FPA (formaldehyde 10%, propionic acid 5%, ethanol 50%, and 35% water) for future use. The collections yielded fresh pollen that was tested for viability, using 0.5% cotton blue in lactophenol. Percentages were calculated after counts were made at 1000× on a Zeiss compound light microscope (Table 2). Grains stained after five minutes were counted as viable. Pollen grains taken from anthers were measured for greatest width at 1000× on a compound microscope. Data were taken from five *Ulmus rubra*, five *U. pumila* and 10 *U.* × *intermedia* anthers, with 15 pollen grains measured per sample (Table 2). Branch-forcing from six *U. rubra*, five *U. pumila* and 22 *U.* × *intermedia* trees yielded flowers from which stamen counts were made (Table 2). The florets of two buds per sample were counted for number of stamens per floret. In April of the third year, fruits were collected and dried from ten *U. rubra*, ten *U. pumila* and 20 *U.* × *intermedia*. Quantitative and qualitative measures and scoring were performed on the five largest fruits present in each sample (Fig. 3, Table 2). The ratios of length to width calculated on the 200 fruits. Scoring of the trichomes was based on a score of (0) for white only and (1) for both rufous and white. The trichomes on the fruits were scored based on presence and density. Essentially glabrous fruits were scored as (0), sparse trichome distribution over the seed on the fruit wall as (1), and dense trichomes that may extend to the wing of the samara as (2).

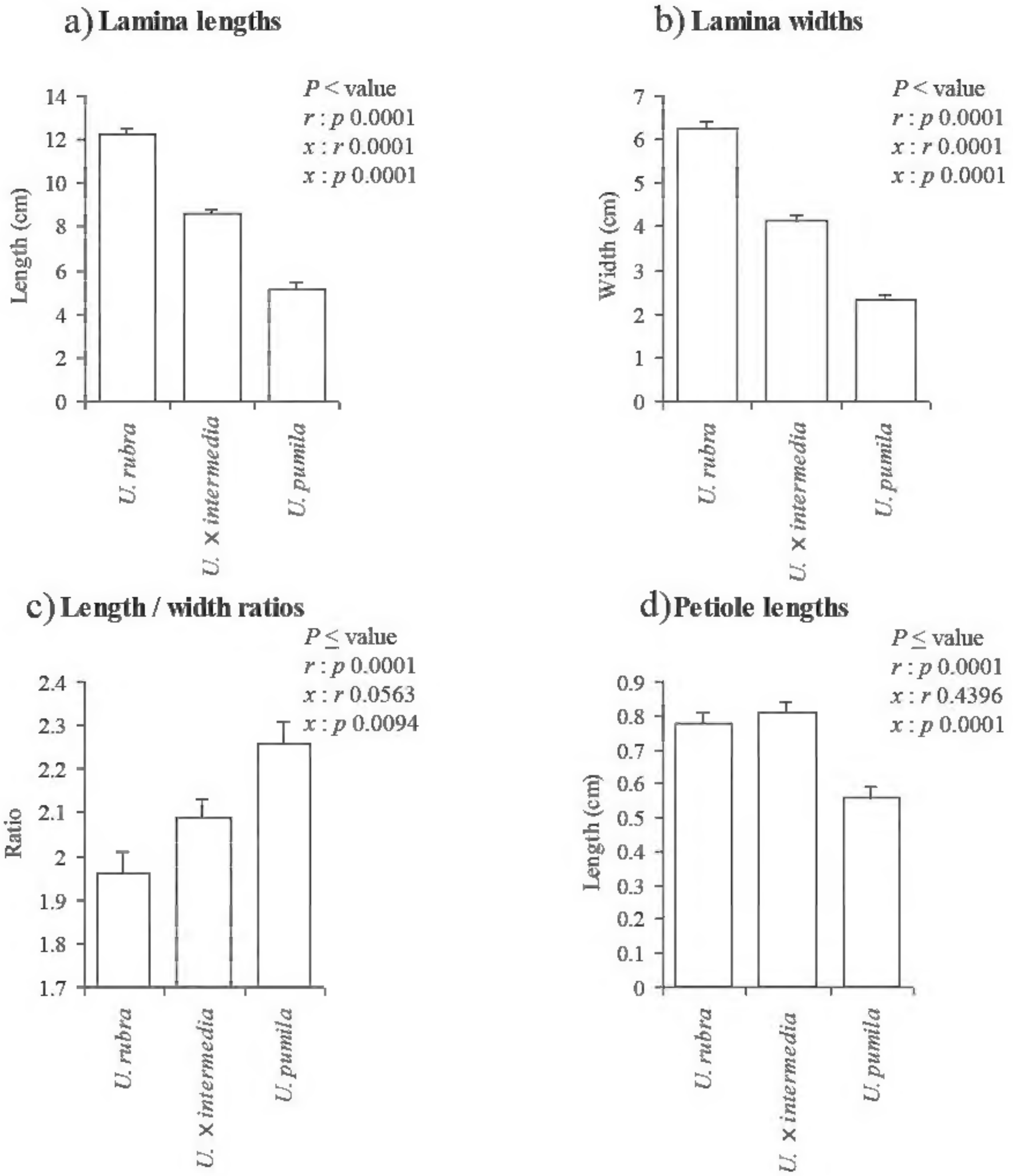
SAS (1999) was used to analyze the data taken from the leaves, buds, flowers and fruits for the 17 measured characters. The quantitative characters, such as measurements, ratios, and counts, were tested using a General Linear Model (GLM) because prior univariate testing supported normal distributions of the data (see Appendix 2 for data matrix). The GLM test detects differences between taxa when data has normal distribution. The least means squares were used for comparisons, with the *P* values indicating differences or lack of differences between taxa. However, this applies only to quantitative data because scored qualitative data are not distributed normally. Chi-square tests tested the five qualitative measures such as color, texture, and distribution. Because 10 characters from leaf and bud samples were available from all 114 trees, cluster analysis was performed using these data solely. The cluster analysis allows the statistical program to cluster points in groups by weighted characters, independent of *a priori* species designations.

RESULTS

Seventeen characters were measured or scored for the three taxa in our study. Thirty-two *Ulmus rubra,* 50 *U.* × *intermedia*, and 32 *U. pumila* analyzed for leaf and bud characters had significant differences between the parental species in all characters ($P<0.05$, Fig. 4a–g, Tables 1 and 2). The hybrids were significantly different from the parental species or were closer to one parental species in a few characters. The lamina lengths and widths segregated all three species ($P<0.0001$), with the mean lamina length of *U. rubra* being the longest, followed by *U.* × *intermedia* and then *U. pumila*. The ratios of lamina length to width between the *U. rubra* and *U.* × *intermedia* ($P=0.0563$) showed no differences but were different when *U. pumila* was compared to *U.* × ($P=0.0094$). The numbers of primary teeth per centimeter were not different between the *U. rubra* and *U.* × *intermedia* ($P=0.1849$) but were different when *U. pumila* was compared to *U.* × ($P<0.0001$). *Ulmus pumila* had the most teeth and *U. rubra* the fewest, with *U.* × *intermedia* intermediate. Because of more primary teeth per centimeter, *U.* × *intermedia* had the most secondary teeth per centimeter, followed by *U. rubra*, then *U. pumila*. *Ulmus* × *intermedia* and *U. rubra* were significantly different ($P=0.0235$), as were *U.* × *intermedia* and *U. pumila* ($P<0.0001$). *Ulmus* × *intermedia* and *U. rubra* were not different in ratio of secondary teeth per primary tooth ($P=0.9771$), but *U.* × *intermedia* and *U. pumila* were ($P<0.0001$), with *U.* × *intermedia* higher than *U. pumila*. A ratio of about 2 secondary teeth per primary tooth held for both *U.* × *intermedia* and *U. rubra*, whereas *U. pumila* had a ratio of one secondary tooth per primary tooth. The two scores of trichomes on the buds were significantly different among the three species ($P<0.0001$). The variation among the parental species and the

hybrid buds varies in bud size, trichome distribution, and trichome color (Table 1). Illustrations summarize the foliar differences among the three species from representative-site collections (Fig. 5).

Canonical correlation analysis of all foliar and bud data yielded a tight cluster for *Ulmus pumila* but a more diffuse cluster for *U. rubra*. *Ulmus* × *intermedia* clustered primarily near or with *U. rubra* (Fig. 6), which is consistent with the lack of statistical differences between *U.* × *intermedia* and *U. rubra*.



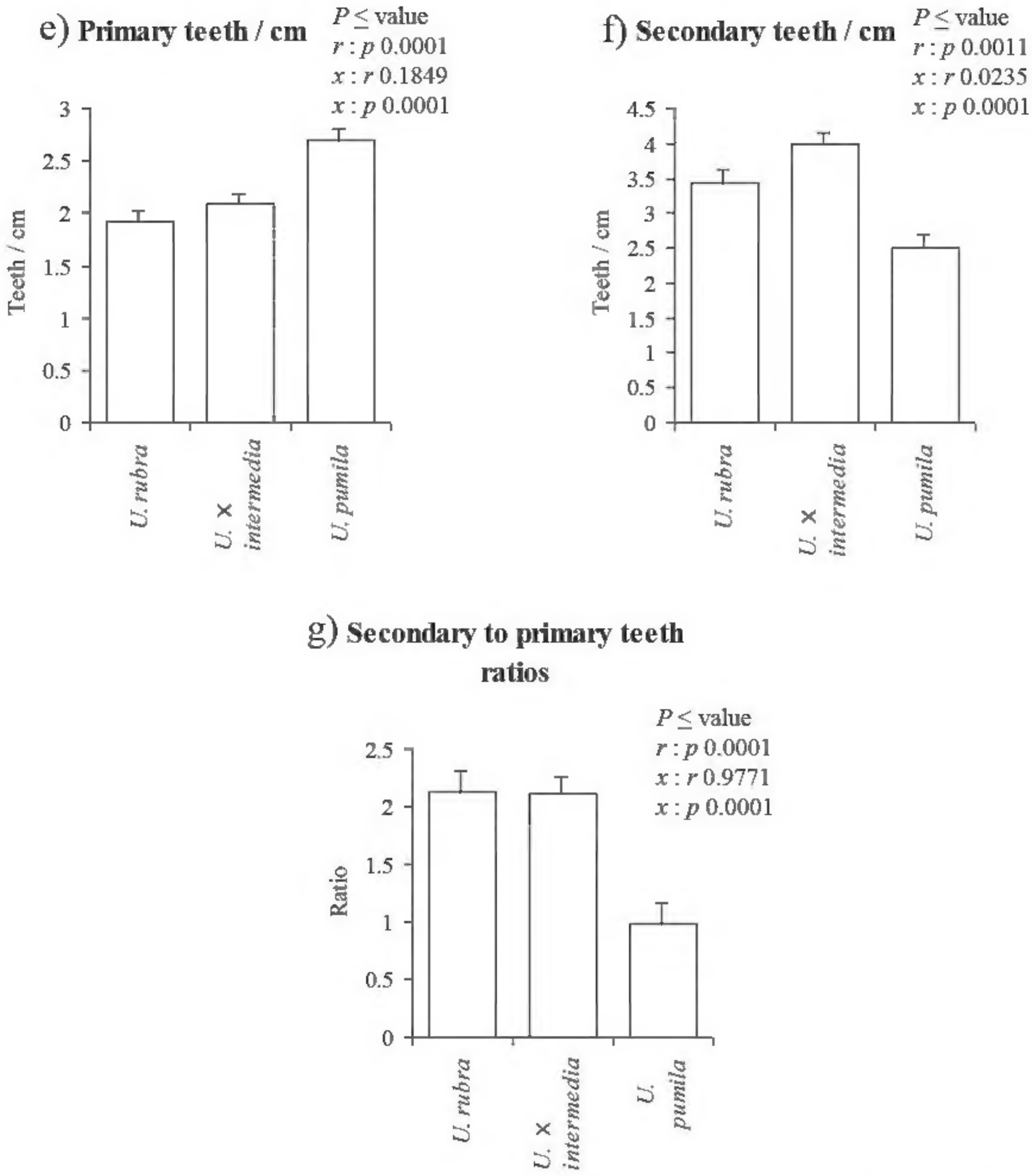


Figure 4. Plots of foliar characters. Presented are the least squares means from 114 trees: *U. rubra* (*r*) = 32, *U. pumila* (*p*) = 32, and *U.* × *intermedia* (*x*) = 50. Bars indicate standard error. Characters: a) lamina lengths; b) lamina widths; c) length/width ratio; d) petiole length; e) primary teeth per cm; f) secondary teeth per cm; g) secondary/primary teeth ratio.

The scanning electron micrographs (SEM) of the adaxial surface of the leaves demonstrate the source of the scabrous or glabrous scores (Figs. 7–9). *Ulmus rubra* data scored as scabrous since the leaves have trichomes nearly three times as large as those of *U. pumila* and *U.* × *intermedia*, which were scored as glabrous. The trichomes of *U. rubra* were just at the range of visibility with a hand lens. The density per area of these larger trichomes is also higher. All *Ulmus* species have glandular and non-glandular trichomes; the non-glandular trichomes are uniseriate and attenuate (Figs. 7–9 and Tobe & Takaso 1996).

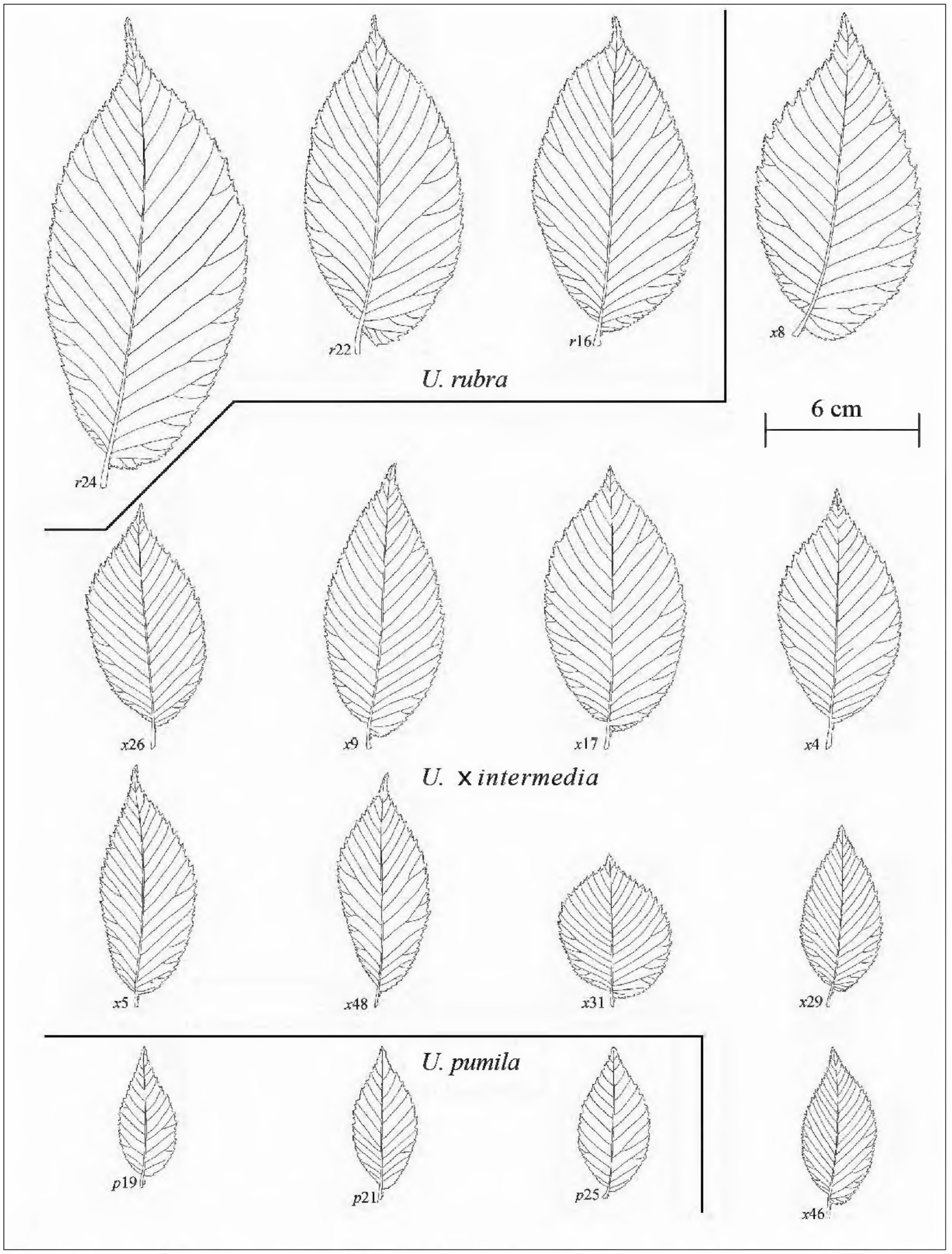


Figure 5. Leaf variation. All leaves at 50% of life size. Specimen numbers are given in lower left of illustrations. Of 13 total sites sampled, 7 sites are represented here.

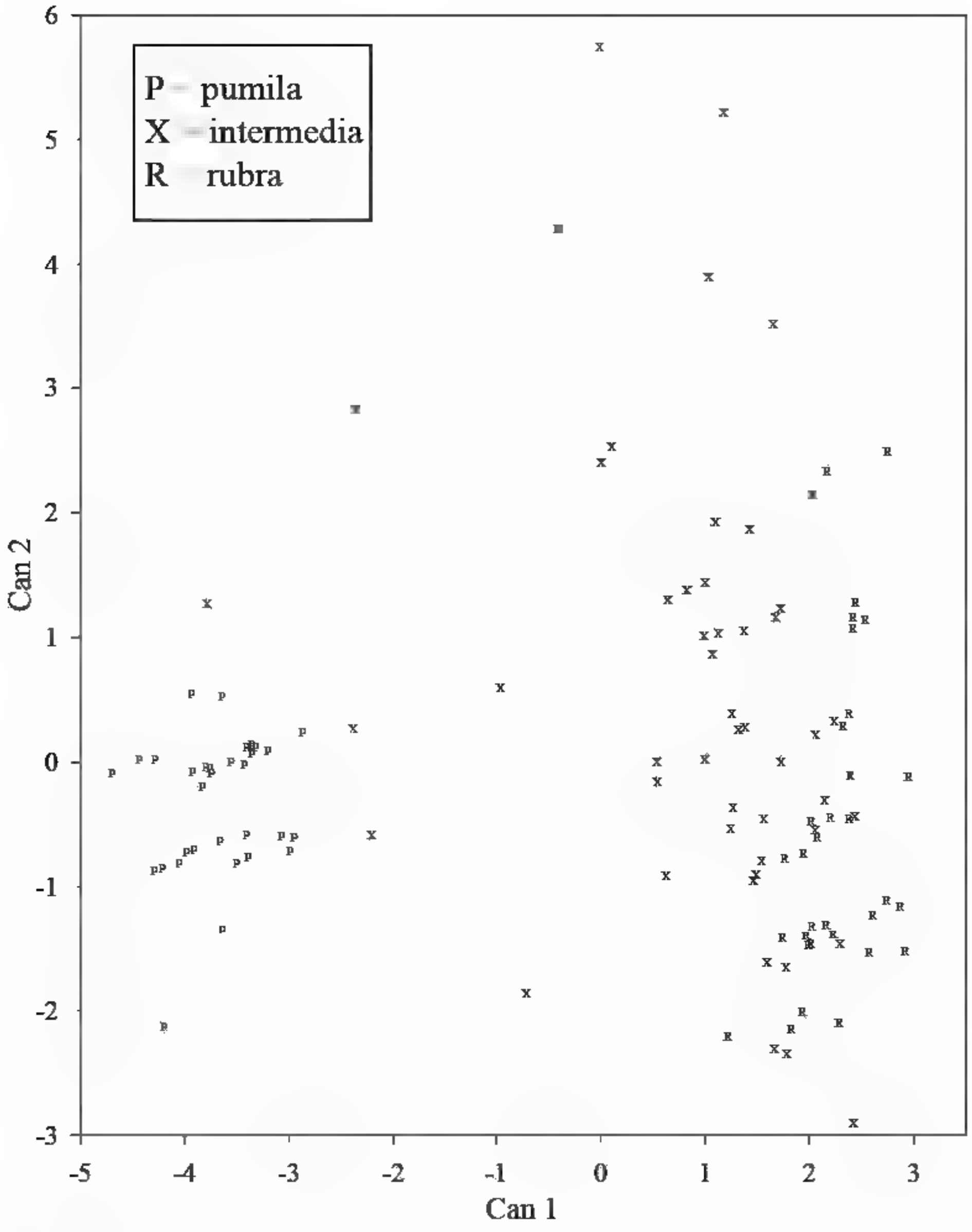


Figure 6 Canonical correlation analysis of all leaf and bud characters. P = *U. pumila*, X = *U.* × *intermedia*, and R = *U. rubra*.

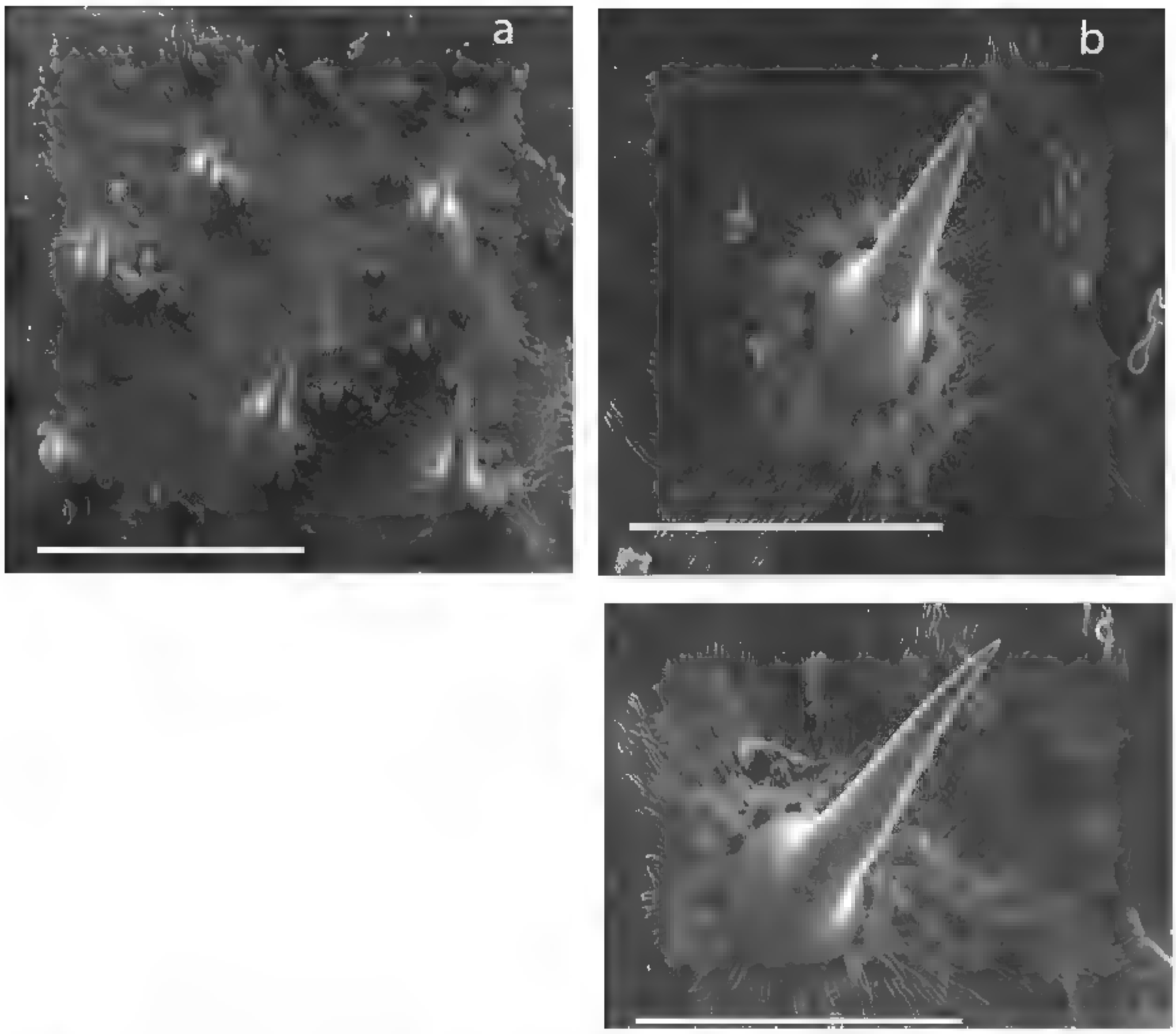


Figure 7 SEM of *U rubra* leaf surface a) Adaxial epidermis demonstrating trichome density, at 107×, scale bar = 500 µm Note two sizes of trichomes b) attenuate uniseriate trichome at 313×, scale bar = 200 µm, smaller trichome, c) 320×, scale bar = 200 µm, larger trichome

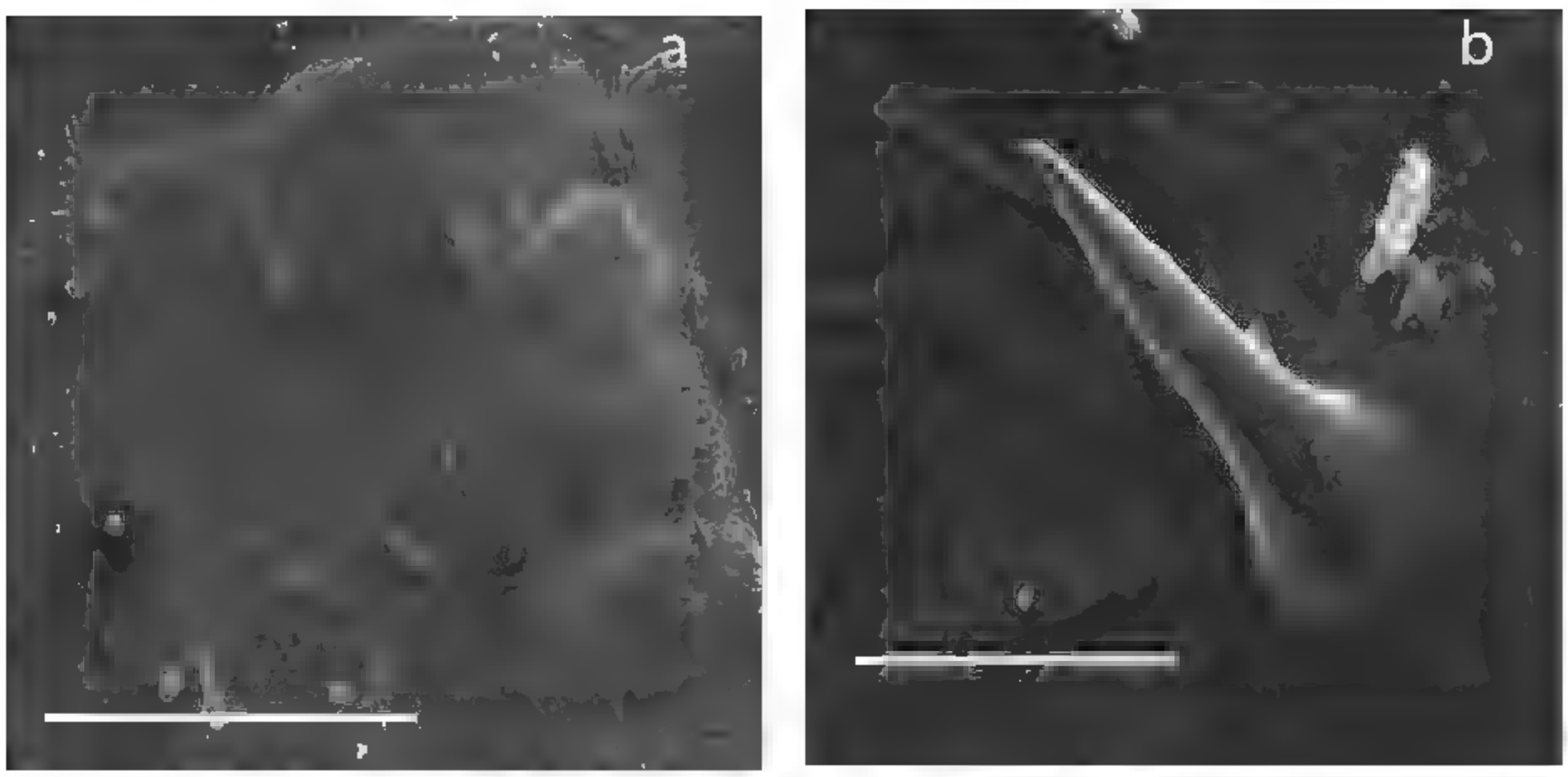


Figure 8 SEM of *U* × *intermedia* leaf surface a) Adaxial epidermis demonstrating trichome density, at 111×, scale bar = 500 µm Note sparse trichomes, this sample is a presumed F_1, and smooth, b) attenuate uniseriate trichome at 1000×, scale bar = 50 µm.

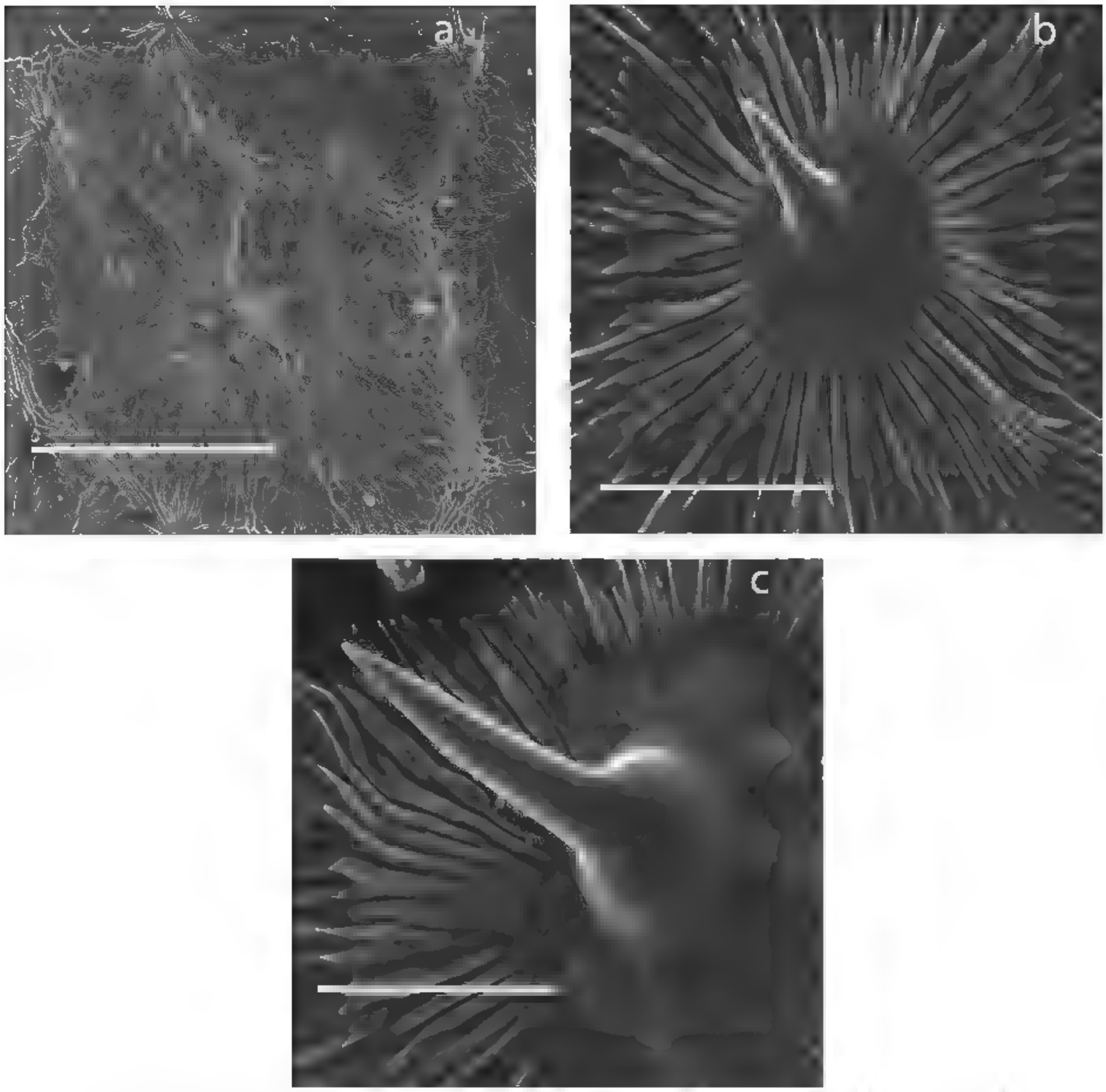


Figure 9 SEM of *U pumila* leaf surface a) Adaxial epidermis demonstrating trichome density, at 100×, scale bar = 500 µm Note two sizes of trichomes b) attenuate uniseriate trichome at 1000×, scale bar = 50 µm, smaller trichome, c) larger trichome

Floral data of stamens per floret yielded significant differences among the three taxa ($P < 0.0001$) *Ulmus rubra* had a mean of seven anthers per floret, *U pumila* had a mean of four, and *U* × *intermedia* was intermediate with a mean of five (Fig 10a) The maximum width measures of pollen were significantly different between the two parental species and *U* × *intermedia* and *U rubra* ($P < 0.0001$, Fig 10b) *Ulmus pumila*, with the smallest pollen, and the hybrid were not significantly different ($P = 0.0575$) An illustration of several florets demonstrates the differences in stamen numbers per floret and in calyces (Fig 11)

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different (P=0 0575) An illustration of several florets demonstrates the differences in stamen numbers per floret and in calyces (Fig 11)

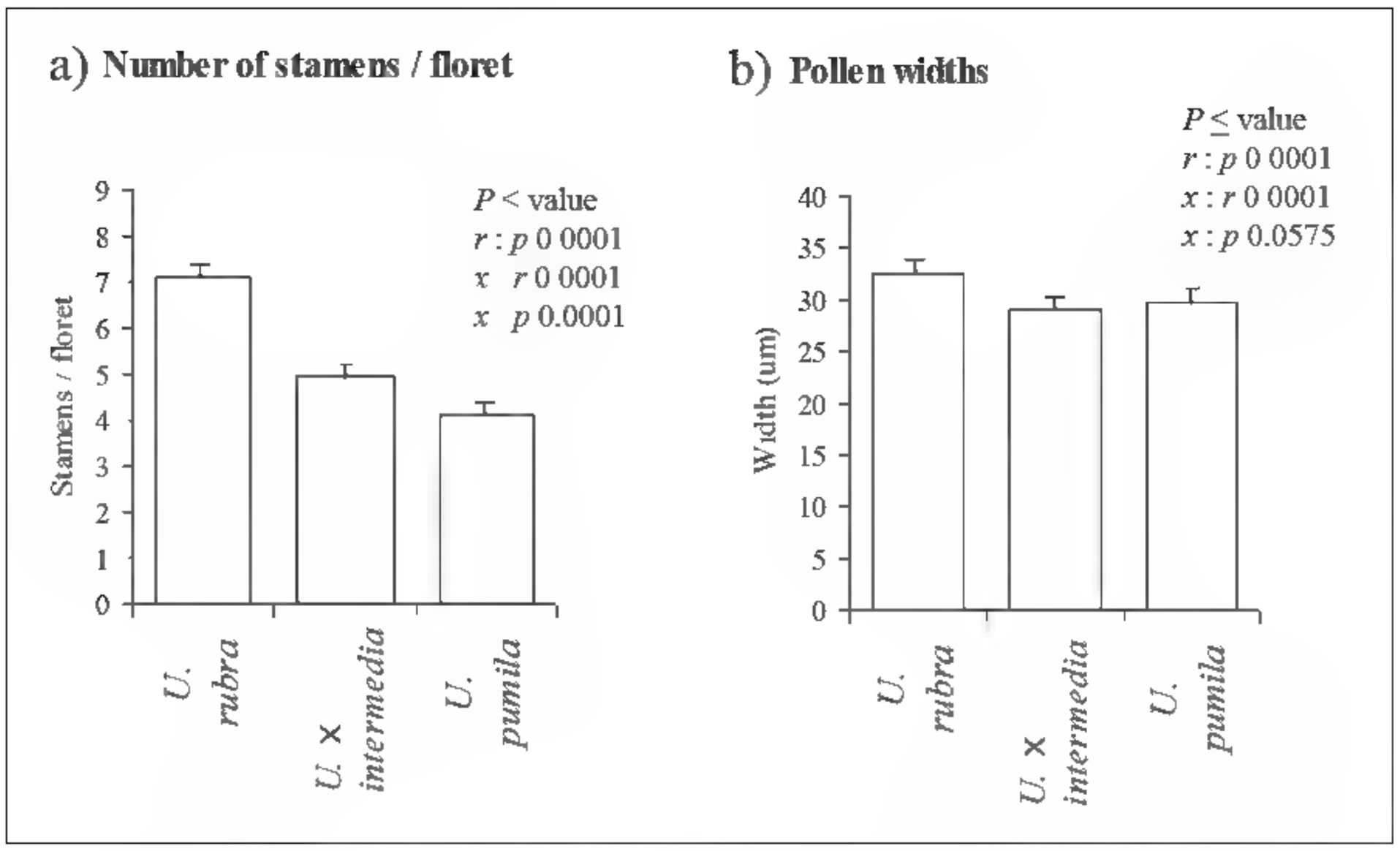

Figure 10 Plots of floral characters a) Stamen numbers Presented are the least squares means from 33 trees *U rubra* (r)=6 and *U pumila* (p)= 5, and *U* ×*intermedia* (x)= 22 with 2 buds per tree measured. N=256, 191, and 947 respectively b) Pollen widths. Presented are the least squares means from 15 trees *U rubra* (r) and *U pumila* (p) =5, and *U* ×*intermedia* (x) =10 with 15 grains per tree measured.

Floral data of stamens per floret yielded significant differences among the three taxa (P<0 0001) *Ulmus rubra* had a mean of seven anthers per floret, *U pumila* had a mean of four, and *U* × *intermedia* was intermediate with a mean of five (Fig 10a) The maximum width measures of pollen were significantly different between the two parental species and *U* × *intermedia* and *U rubra* (P<0 0001, Fig 10b) *Ulmus pumila*, with the smallest pollen, and the hybrid were not significantly different (P=0 0575) An illustration of several florets demonstrates the differences in stamen numbers per floret and in calyces (Fig 11)

The florets produced stigmas after anthesis The stigma branches on *Ulmus rubra* were dark red or magenta from their emergence to desiccation several days later, and the colors persisted in the developing fruit *Ulmus pumila* stigma branches were only white, the color persisting into early fruiting *Ulmus* × *intermedia* demonstrated hybrid intermediacy by having variously white, pink, or red stigma branches In the samples observed, some trees had only white, only red, or only pink stigmas A few samples had combinations of these colors, not only on the same branches but also on the same stigmas This character trait was consistent from stigma emergence until desiccation

Branches collected on the same day and forced into flowering in the laboratory demonstrated differential flowering *Ulmus pumila* flowered first, sometimes 18 hours after collection *Ulmus rubra* began anthesis as the stigmas of *U pumila* emerged and pollen-drop had ceased, a 3 7 day

delay. *Ulmus* × *intermedia* samples began anthesis as early as 12 hours after *U. pumila* or delayed as much as 3 days. The temperatures of the air and water were constant (21°C) between samples, usually with mixed species in the same bucket of water. Thus the flowering sequence was *U. pumila*, *U.* × *intermedia*, and then *U. rubra*.

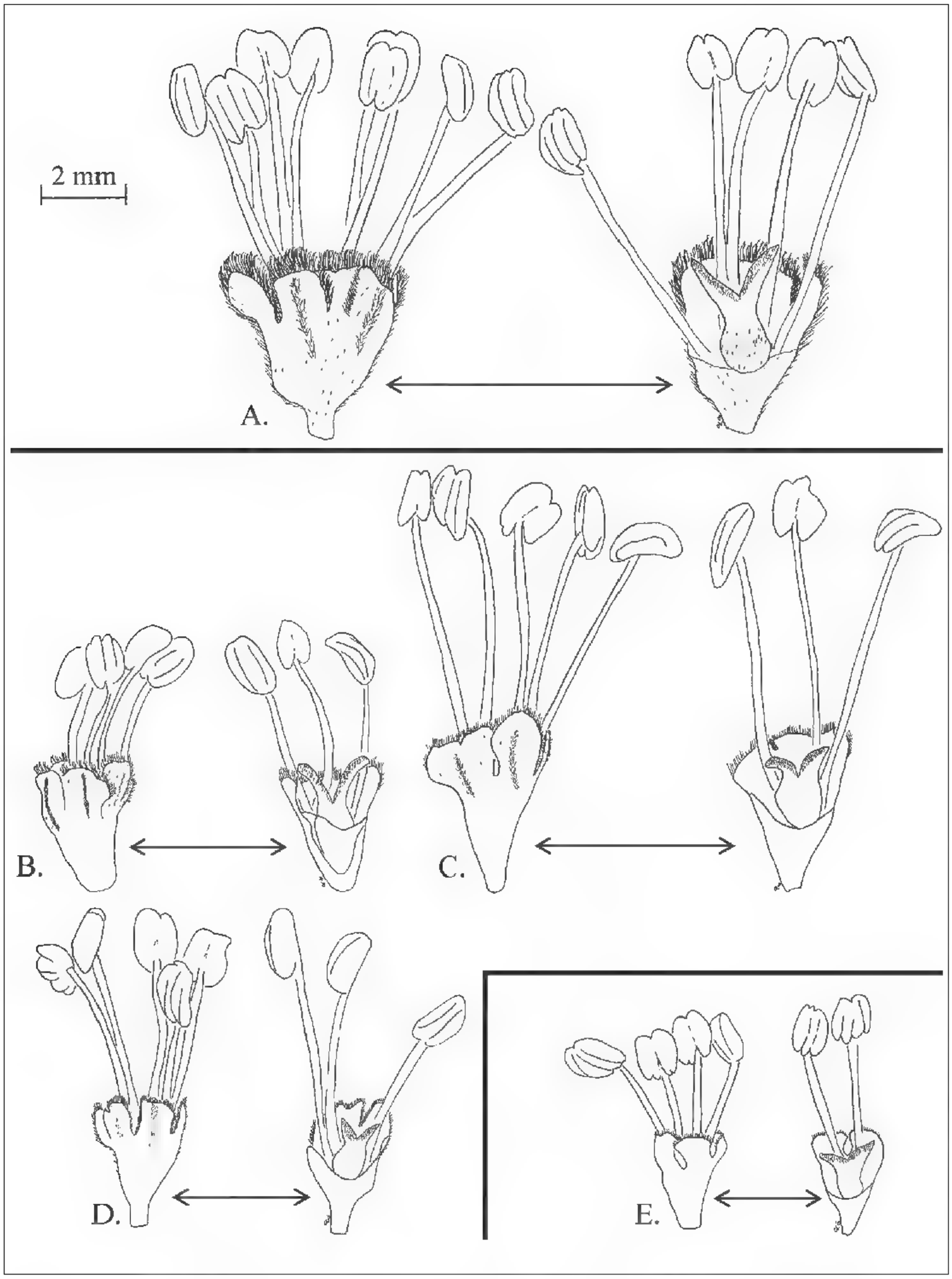

Figure 11 Variation of floral characters A *U. rubra*, B-D *U* × *intermedia*, E *U. pumila* Images on right show cut-away calyx, revealing the pistil

In two years of our study, we tested fresh pollen for viability Two *Ulmus rubra* samples were sampled for 673 pollen grains, of which 661 were stained, indicating viability of 98 2% Three *U. pumila* were sampled for 793 grains, of which 742 were viable, yielding 93 3% viability *Ulmus* × *intermedia* was similarly sampled, with 1,704 of 1,812 grains staining, for a 94 0% viability

The fruit analysis yielded primarily significant differences Five fruits were selected from each of 10 trees of *Ulmus rubra,* 20 of *U* × *intermedia*, and 10 of *U. pumila* The width, length-to-width ratio, and color and position of the trichomes were significantly different among the three species ($P<0.0001$) The length of the fruits was not significantly different between *U. rubra* and *U. pumila* (P 0 0971), but it was between the hybrid and parental species ($P<0.0001$, Fig 12) The variation in the fruits among the two parental species and the hybrids differed in size, color, and pubescence The presumed F_1 hybrids have fruits that are much larger than those of the parental species

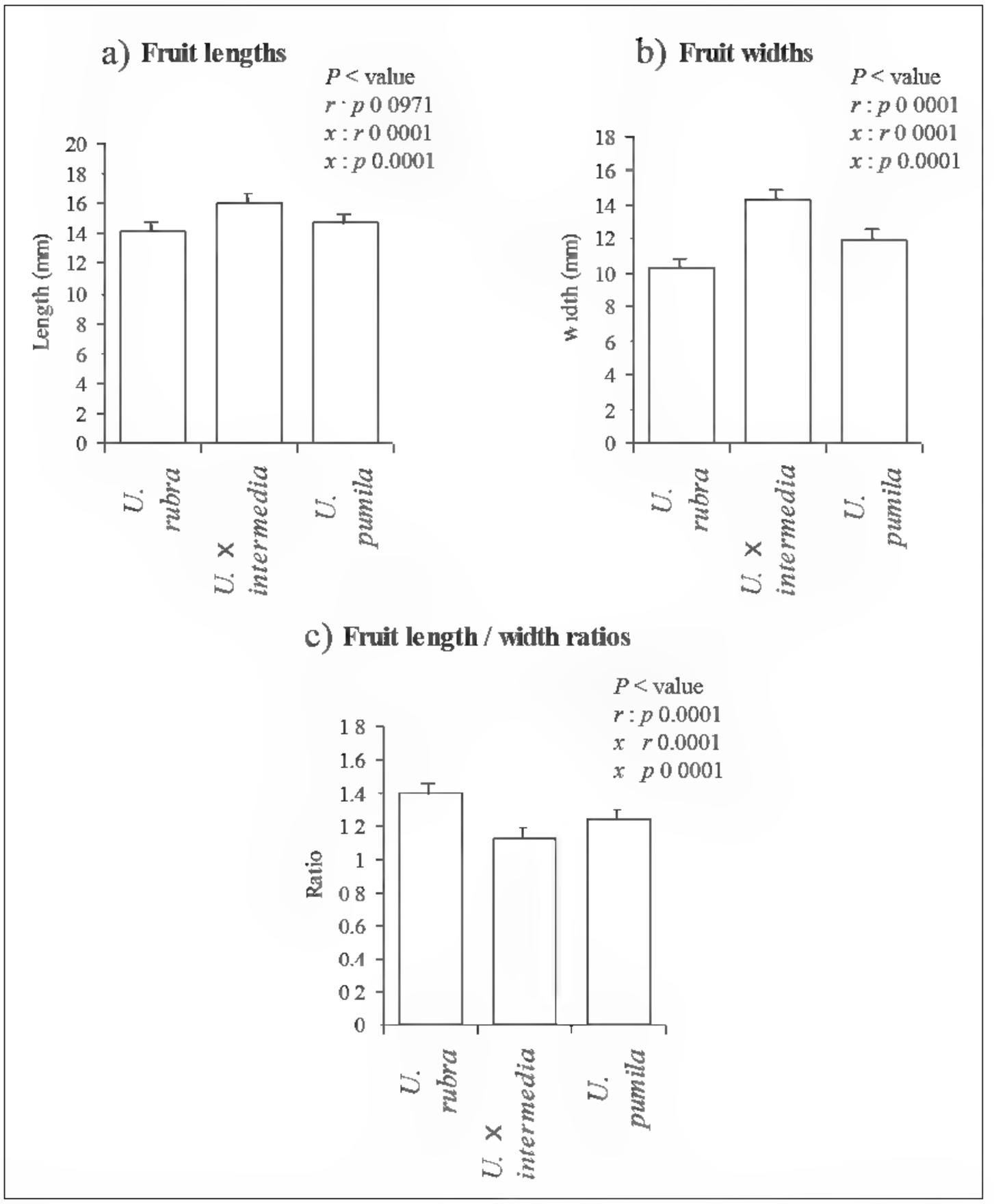

Figure 12 Fruit characters Presented are least squares means from 40 trees *U rubra* (*r*) and *U pumila* (*p*) 10, and *U* × *intermedia* (*x*) 20 with five samples from all trees taken Bars indicate standard error a) Fruit length, b) fruit width, fruit length width ratio

Ulmus rubra fruits have dense trichomes over the seed within, but few trichomes had the rufous color often given as a key character. That color comes mostly from the the epidermal cells of the pericarp itself. In the ten *U. rubra* trees sampled, few to all fruits demonstrated this color. One sample of *U.* × *intermedia* had rufous epidermal coloring on all fruits sampled. Sparse or no trichomes were noted on hybrid samples.

A summary of the results of the foliar and bud characters based on the original 114 trees is provided (Table 1). A summary is also provided of the stamen, pollen, and fruit characters, based on sub-samples of the 114 trees (Table 2). Significant differences were found among all seventeen characters of the three species measured.

The foliar and reproductive phenologies of the three species are overlapping, not synchronous. Onset of spring in southeastern Nebraska ranges from late January to March, but once temperatures stay above 0° C for several days, flowering begins. *Ulmus pumila* flowers first, followed by *U.* × *intermedia* demonstrating primarily *U. pumila* traits, then by *U.* × *intermedia* demonstrating primarily *U. rubra* traits, and lastly by *U. rubra*. This same trend continues through fruit set and maturation. Foliar flushing began as the fruits drop in all three species. The delay then becomes most apparent because it is common to find *U. pumila* with leaves at 2–3 cm before any leaves appear on *U. rubra*.

A summary of this study's results is provided below for taxonomic purposes in the form of a synoptic key. The key is based on all quantitative and qualitative data reported in the previous text and figures. The descriptions summarize only the data from this study and are not based on material from trees growing outside our study area.

1 Leaves semi-conduplicate, 8–17 × 4–8 5 cm, ovate to obovate, harshly scabrous (trichomes visible with hand lens), large tufts of hairs in axils of veins, margin primarily triply serrate, apex acuminate, floral buds over 2 3 rufous-pubescent, leaves drying with dull lustrous silver cast ——— **Ulmus rubra**
1 Leaves not conduplicate, not harshly scabrous, tufts in axils of veins small, buds primarily glabrous

2 Leaves 4–7 × 1 5–3 5 cm, elliptical to lanceolate, glabrous, doubly serrate, rarely singly, apex acute, floral buds tiny, brown, black, or dark purple, scale margins pubescent with white trichomes, leaves drying to dull green ——— **Ulmus pumila**
2′ Leaves 4 5–12 × 2 5–6 cm, ovate to lanceolate, scabrous to glabrous, margin primarily triply serrate, apex acuminate to acute, scales of floral buds usually rufous marginally, basal scales purple, brown and glabrous, fully pubescent or marginally pubescent with red, white or red and white trichomes, leaves drying with a dull, lustrous silver cast ——— **Ulmus × intermedia**

Ulmus rubra Muhl. Red or Slippery Elm

Leaves conduplicate, often dark green with silver cast abaxially, 8–17 × 4–8.5 cm, petiole 0.5–1.2 cm, ovate to obovate, scabrous (trichomes visible with hand lens), large tufts of hairs in axils of veins; margin primarily triply serrate (2 denticles per primary tooth), apex acuminate, base oblique. Leaves drying with a dull lustrous silver cast, difficult to press due to conduplicity. Floral buds over 2 3 rufous-pubescent; bud scales dark purple–brown with rufous and white trichomes, vegetative buds likewise, with occasional glaucous appearance. Florets subsessile, appearing in February–March, pubescent with rufous trichomes; stamens 5–9, usually 7; stigmas exsert, deep red. Pollen typical for genus, maximum width 27.0–42.0 (32.6) µm. Samaras 10–17 mm × 6.5–13 mm, cream or brown, white-pubescent over seed on pericarp, extending to mid-rib of fruit and sparsely to samara wings, pericarp over seed usually red, its margins glabrous.

Ulmus pumila L. Siberian Elm

Leaves 4–7 × 1.5–3.5 cm, petiole 0.3–1.0 cm, elliptical to lanceolate, drying to dull or bright green, glabrous, doubly serrate (1 denticle per primary tooth), rarely singly, apex acute, base slightly oblique. Floral buds tiny, brown, black, or dark purple, scales white-pubescent on margins. Florets subsessile, appearing in February–March (the first elm to flower in any year), the calyx with marginal white pubescence; stamens 4; stigmas exert and white–light green. Pollen typical for genus, maximum width 22.0.0–35.0 (29.7) µm. Samaras 12–19 × 9.5–15 mm, cream to white, glabrous.

Ulmus × intermedia Elowsky, **hybr. nov.** **TYPE: USA. Nebraska**. Lancaster Co.: Saltillo township, Wagon Train Lake State Area, 40.6262169° N, 96.5786938° W, tree 10 m tall, trunk 35 cm diameter, 9 Sep 1998, *C.G Elowsky x48* (holotype: NEB 318094).

Hybrid swarm of *Ulmus rubra* and *Ulmus pumila*, demonstrating ranges of characters between the parental species. Leaves 4.5–12 × 2.5–6 cm, petiole 0.3–1.2 cm, blade ovate to lanceolate, scabrous to glabrous, drying with a dull silver cast, margin triply serrate (2 denticles per primary tooth), apex acuminate to acute, base oblique, abaxial tufts larger than in *U. pumila*. Floral budscales usually rufous marginally, basal scales purple, brown, and glabrous, fully to only only marginally pubescent with rufous or white hairs. Florets subsessile, appearing February March in the study area and between *U. pumila* and *U. rubra*; perianth from pubescent with rufous hairs to marginally with white trichomes; stamens 4–7, usually 5; stigmas exserted, white, pink, red, or any combination. Pollen typical for genus, maximum width 23.0–35.0 (29.0) µm. Samaras 11.5–21.0 × 10.0–20 mm (F_1 fruits larger than parental species), cream to white, rarely red or pink on pericarp over seed, glabrous to sparsely pubescent over seed.

Representative specimens of other members of this swarm are NEB 318091 318101.

DISCUSSION

Phenotypic intermediacy between the putative parents is the first obvious indication of a hybrid. As summarized in Tables 1 and 2, several of the characters measured are intermediate in *Ulmus × intermedia,* including lamina length, width, and length-to-width ratio; number of primary teeth per centimeter; trichome color and distribution on the buds; and number of stamens per floret and density of trichomes on the pericarp. Intermediacy was also noted in flowering, fruit drop, and leaf flushing. Other characters are less easily characterized as intermediate. Dominance by some characters is demonstrated by both parental species in this hybrid. Fruit color, leaf texture, and trichome density on the fruit wall and the buds seem to be controlled by *U. pumila*. The number of secondary teeth on each primary tooth, and the color of the leaf after drying are apparently controlled by genes of *U. rubra.* The other characters are independent of either parent, such as pollen width, petiole length, and the size of the fruits. A majority of those characters measured are intermediate, so phenotypic intermediacy is supported for *U. × intermedia*. This intermediacy is demonstrated with scatter diagrams produced from canonical analysis (Fig. 6).

Reduced fertility is often evidence of hybridization, but we did not detect that in *Ulmus × intermedia*. Viability of pollen in this study was over ninety percent in the hybrids. In the spring of the third year, a majority of the hybrids sampled were heavily weighed with fruits, out-producing all *U. rubra*, and a few fruits sprouted in storage. These observations, in addition to those conducted by Kaul (1995), where pollen and fruit viability were over ninety percent for *U. × intermedia*, do not indicate fertility reduction. Furthermore, Collins (1967) performed artificial hybridization of *U. rubra* and *U. pumila* and demonstrated no reduction of fertility in the F_1 or F_2 generations.

Segregation of the F_2 is another indicator of hybridization. All samples of *Ulmus* × *intermedia* were within the expected range of a hybrid swarm for the measured characters. Only sample *x*31 (Fig. 5) was abnormal for the group, being orbicular in leaf shape, a trait demonstrated on the whole tree. This one variant aside, nothing resembling segregation was noted in this study.

Overlapping geographic distribution is another test for a putative hybrid. Because *Ulmus pumila* is widespread as a naturalized weed, and because *U. rubra* has a native range east and west of the sample area, the question of overlapping distribution is a moot point. *Ulmus pumila*, an Asian introduction, has been artificially brought into contact with *U. rubra*, yielding sympatry that gives rise to the hybrid *U.* × *intermedia*.

Hybridization is often tested by its ability for artificial resynthesis. While that test falls outside the range of our study, artificial resynthesis has been done elsewhere by other researchers (Smalley & Guries 1993; Santamour & Bentz 1995). The major purpose for hybridization has been to circumvent the DED pandemic because *Ulmus pumila* is resistant but is an undesirable landscape tree.

Detection of hybrids by molecular methods highly complements the morphological methods shown here (Twyford & Ennos 2012). One classical chemotaxonomic study of flavonoids is available for *Ulmus rubra* and has been used for sectional treatments of *Ulmus* (Sherman 1987) and could be used for the investigation of *U.* × *intermedia*. Flavonoids of a few samples of this study were isolated and distinct patterns between sugar moieties on the flavonoids of the three species were found (data not shown) and agree with the work by Sherman (1987). Chloroplast DNA also has been studied in native *Ulmus* of North America for taxonomic sectional analysis (Wiegrefe et al., 1994). The use of microsatellites in that hybrid swarm and the population genetics of the parents give strong evidence for the presence of this hybrid in North America and indicate introgression (e.g., Zalapa et al. 2008a, 2009, 2010).

Hybridization is also indicated by the ecological intermediacy, which is commonly demonstrated in other hybrid species (Anderson & Hubricht 1938; Maze 1968; Chechowitz et al. 1990; Grant & Wilken 1988). While *Ulmus rubra* is a species of mesic habitats and *U. pumila* is from the dry steppes of China and Siberia, they both appear anywhere in our study area that has somewhat undisturbed ground, including parks, roadside ditches, empty lots, and abandoned farms. Ecological isolation does not occur between the two parental species and therefore not with *U.* × *intermedia*. This trend may not be true at the edges of the *U. rubra* range to the west of the study area.

The phenotypic intermediacy, overlap of distribution, and artificial resynthesis of the hybrid are seen with these data. The reduction of fertility, segregation of the F_2 generation, and ecological intermediacy do not hold true for all known hybrids of other species and are not observable in our study population.

Ulmus × *intermedia* demonstrates all the trends associated with a hybrid swarm. Characters span the range from one pare ntal species to the other (Figs. 5, 11). Sample *x*8 has leaves very similar to *U. rubra,* and sample *x*46 has leaves similar to *U. pumila*. The only noted differences between *x*8 and *U. rubra* are the difference in bud pubescence and the acuminate apex on the leaf. Sample *x*46 has characters matching those for *U. pumila*, except the two secondary teeth on each primary tooth, and the dull lustrous silver cast all *U. rubra* and *U.* × *intermedia* samples demonstrate after drying. These two samples may make an unlikely case for introgression, but given the strong evidence of Zalapa et al. (2010) for introgression, we cannot dismiss the possibility.

Ulmus × *intermedia* is more likely to be a hybrid swarm in the very early stages of introgressing (e.g., decades of generations). This correlates well with the known introduction time of *U. pumila* into North America in 1908 (Webb 1948; Wyman 1951). By 1928, *U. pumila* was still

uncommon, being planted primarily on research stations (Dickens et al. 1928). It was not until the 1940s and 1950s that it became overly planted (Anonymous 1950; Webb 1948). The tree was not mentioned in earlier floristic works for Nebraska (Webber 1890; Petersen 1912, 1923; Pool 1951), and it was noted only as a cultivated tree in the state by Winter (1936). The University of Nebraska–Lincoln herbarium (NEB) has no specimens collected before 1961. The time and number of generations for introgression to occur is unknown, but roughly 70 years have passed since hybridization began in our study area. A generation time of ten years for trees equates to seven generations of hybrids. Given the results of Zalapa et al. (2010), it appears that seven possible crosses are enough for the hybrid swarm to display introgression.

The isolating factors required for introgression are not present where sympatry occurs, but nevertheless introgression may be occurring in Nebraska and other areas. Areas where *Ulmus rubra* is at the edge of its natural range may provide gene flow to *U. pumila* populations. The western edge of *U. rubra*'s range is only a few km west of our study area, and *U. rubra* genes could move beyond this range into *U. pumila* populations, which extend farther to the west. *Ulmus rubra* is a species of mesic habitats, but its wind-borne pollen is not restricted to those environments – hence a possible mechanism for introgression. Additionally, we have shown here the viability of *U.* × *intermedia* pollen, another possible mechanism for introgression. Any location where *U. rubra* was once sympatric with *U. pumila* is a possible location for introgression if *U. rubra* has been removed. Since *U. pumila* is such an aggressive weed, it is highly unlikely to have been removed from any region it has invaded. Because *U. rubra* is less productive with fruits and highly susceptible to DED, its demise is a possibility. In light of this study, the introgressant *U. pumila* would most likely have leaves with 2 teeth per primary tooth, anatomy that conveys the dull silver color upon drying, 4 to 5 stamens per floret, and synchronous flowering with the non-introgressed *U. pumila*. Due to the young nature of this introgressant hybrid swarm, these characters are not yet observed.

Eventually, *Ulmus rubra* could be hybridized out of existence (Schlautman et al. 2012), accelerated by its great susceptibility to Dutch elm disease. *Ulmus pumila* or *U.* × *intermedia* are within sight of *U. rubra* at most sites sampled, making pollen transfer between species easy among these anemophilous trees. *Ulmus pumila* and *U.* × *intermedia* also fruit more prolifically, and whereas fruits per tree of *U. rubra* number in the hundreds, the same-sized trees of *U. pumila* or *U.* × *intermedia* can have fruits in the tens of thousands. The ecological impact is yet unmeasurable, but *U. rubra* is a species known to have foliar domatia that act as foliar protection for beneficial mites (Willson 1991). Because *U. pumila* and *U.* × *intermedia* both have reduced axillary tufts of hairs, these mite species may be absent from their habitat as well. The extent or importance of any one species in an ecosystem is difficult to gauge, but its loss may cause further losses. As demonstrated before in other systems, anthropochores can cause devastating losses or changes in native ecosystems.

The foliar dentition of *Ulmus pumila* has been reported as singly serrate (e.g., Sherman-Broyles et al. 1997), but *U. pumila* rarely has single serration in this sample area. Whether this is a variation specific to Nebraska, the Great Plains, or the whole of North America is a question that must be addressed. Other such basic morphological mistakes have been noted in this study, concurrent with the lack of information available on these species. *Ulmus rubra* fruits are reported as having rufous trichomes, but in our local trees the coloration is from the pigmented fruit wall.

The amount of variation found in the small geographic area we studied was unexpected. Localized sites of introgression may also then be detected. The extent of damage to *Ulmus rubra* may also be gauged with much wider sampling. A larger area of sampling to test would resolve many of the questions raised in this study. Such research should be performed before another fifty years pass and the elms of America are as difficult taxonomically as the elms of Europe. The possibility exists that syngameons may occur where *U. pumila, U. rubra, U.* × *intermedia,* and *U. thomasii* are

sympatric, but only if *U. thomasii* hybridizes. Syngameons would mean a hybrid swarm of several species, causing homogenization and raising interesting ecological questions.

Two other elms occur naturally in or near our study area: American elm, *Ulmus americana* L., and rock elm, *U. thomasii* Sarg. American elm is still abundant despite the ravages of more than fifty years of DED (Hubbes 1999). Natural hybridization with *U. pumila* has been reported elsewhere (Santamour 1970), but we have not detected it in our area, where its flowers emerge later than those of our study species. All elms studied globally thus far have a diploid number of $2n = 28$, except *U. americana* is mostly tetraploid at 4n = 56 (Sax 1933; Elias 1970; Santamour 1993; Santamour & Ware 1997), but recently 20% of trees in eastern states were found to be diploid and have been identified as a polyploid complex (Whittemore & Olsen 2011). Another barrier to hybridization in *U. americana* is pollen incompatibility—either the pollen fails to develop fully functional pollen tubes, or the pollen from other species fails to do the same on receptive *U. americana* stigmas (Ager & Guries 1982; Bob et al. 1986).

Ulmus thomasii is 50 mi (80 km) away from our study area, seemingly distant enough to be irrelevant to our study (Kaul et al. 2011), and its flowers emerge much later in the season than those of our study plants, although *Ulmus pumila* is reported to cross naturally with it elsewhere (Graves 1948). *Ulmus thomasii* and *U. rubra* are reported to hybridize (Scholz 1965); Hess and Dunn (1967) believed the ecological isolation of those species might not allow viable offspring to survive outside urban areas of cultivation, hence the lack of detection of naturally occurring hybrids.

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